REMARKS

Favorable reconsideration and allowance of the present application are respectfully requested in view of the foregoing amendments and the following remarks.

Claim 4 has been canceled without prejudice or disclaimer. Claims 1-3 and 5 have been amended to more clearly point out the invention or to correct grammatical errors. Accordingly, claims 1-3 and 5 are currently pending and are believed now in the condition of allowance.

Correction of the Form PTO-1449 is acknowledged.

The specification has been amended to comply with 37 CFR §§ 1.821 et seq. Corrected Figs. 6a-6b incorporating the appropriate sequence identifiers are submitted herewith. The sequence entry for *M. terrae* has been deleted. Approval of the corrections and entry of the corrected drawings are requested.

The specification stands objected to because of trademarks which were not capitalized: DYNAZYME®, GENECLEAN®, and BIODYNE® have been amended.

As noted above, the claims have been amended to correct misspellings and grammatical errors. Withdrawal of the claim objections is requested.

Claim Rejection under 35 U.S.C. § 112

Claims 2-5 stand rejected under 35 U.S.C. §112, first paragraph because they are allegedly not enabled for methods employing any "DNA fragment from microorganism to identify." The Examiner, however, recognizes that claims 2-5 are enabled for methods of identifying the species and/or subspecies of a mycobacterial strain in which the DNA fragment to be tested corresponds to the rpoB gene.

Claim 2 has been amended to recite specific restriction enzymes that may be used in the digestion of DNA fragments to obtain DNA fragment length polymorphism patterns in steps a) and d) as well as to limit the sources of DNA fragment to be identified. Claim 2 has been further amended to specifically recite that the rpoB region of the DNA fragment is amplified using specific primers.

Claims 2-5 stand rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite. The claims have been clarified as shown above.

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All the changes are made for clarification and support for the changes may be found in the application as originally filed. Withdrawal of the Section 112 rejections is requested.

Claim Rejection under 35 U.S.C. § 102

Claims 2-5 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Lee et al. This rejection is overcome by establishing a priority date before the publication of Lee et al. Attached is a certified translation of priority document 1999-46795 (Republic of Korea) filed October 27, 1999.

Withdrawal of the Section 102 rejection is requested.

In light of the above amendments and remarks, Applicants respectfully submit that pending claims 1-3 and 5 as currently presented are now in condition for allowance. Early notice of such action is earnestly solicited and will be greatly appreciated. If any outstanding issues remain, the Examiner is invited to contact the undersigned at the telephone number provided.

Respectfully submitted,

NIXON & VANDERHYE P.C.

Bv.

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APPENDIX MARKED-UP VERSION TO SHOW CHANGES

IN THE SPECIFICATION

The specification is amended as follows.

Paragraph spanning pages 12-13:

The primer set used to amplify the region of the rpoB were PCR amplification. 5'-TCAAGGAGAAGCGCTACGA-3' (RPO5', SEQ ID NO:25) and 5'-GGATGTTGATCA GGGTCTGC-3' (RPO3', SEQ ID NO:26) resulting in about 360-bp PCR product (base number 902 to 1261 and codon number 302 to 420 based on the sequence numbers for the rpoB gene of M. tuberculosis, [GenBank accession No. p47766]). The primer sequences were selected from the region of the rpoB genes that have been previously identified from M. tuberculosis, M. leprae, and M. smegmatis (12, 13, 22). The primers were made to amplify the region between the variable region and conserved region based on the genetic information for the rpoB gene of Escherichia coli. As a result, PCR products included 171-bp of variable region and 189-bp of conserved region. Variable region was amplified in this experiment based on an assumption that the polymorhphic nature of this region might help the clear distinction of each mycobacterial species using molecular biological techniques such as PRA and PCR-DNA hybridization. On the other hand, the region of the rpoB gene was also chosen to be flanked by highly conserved sequences, thus can be suitable for PCR amplification of the rpoB region of all mycobacterial species using the same set of PCR primers.

First full paragraph at page 13:

PCR was carried out in a final volume of 50 μ l with 10 μ l of DNA supernatant containing approximately 10 ng of genomic DNA, 10 pmole of each primers, 2 mM MgCl₂, 200 μ M of deoxynucleotide triphosphates, and 1 unit of DyNAzymeTMII DYNAZYME® II DNA polymerase (FINNZYMES, Espoo, Finland). DNA samples were first denatured completely by incubation at 94°C for 5 min before amplification cycle,

then amplified using a cycle that includes (1) denaturation at 94°C for 1 min, (2) primer annealing at 58°C for 1 min, and (3) elongation at 72°C for 1 min for 35 times using a Thermocycler thermocycler (model 9600, Perkin Elmer). After the last amplification cycle, the samples were incubated further at 72°C for 7 min for complete elongation of the final PCR products. Positive and negative controls were always included in each PCR reaction. The positive control was the PCR mix with DNA of reference strain, *M. bovis*, and the negative control was the PCR mix without any DNA. After the PCR, the amplification results were visualized using 1.5% agarose gel electrophoresis and ethidium bromide staining.

Paragraph spanning pages 14-15:

Cloning and sequence analysis. For sequence analysis, PCR products were purified by using a Geneclean GENECLEAN® kit (BIO101, Vista, Calif. USA) from an agarose gel and cloned into TOPO-TA cloning vector (Invitrogen Co., Carlsbad, CA) by the method recommended by the manufacturer. DNA sequencing was done by the dideoxy nucleotide-chain termination method (21) using ARL automatic sequence (Pharmacia Biotechs, Uppsala, Sweden). For each clone, M13 reverse primer and T7 promoter primer were used separately to read sequences from both directions. Sequences were aligned using a multiple sequence alignment program (6).

Table 2 spanning pages 15-16:

Table 2. Oligonucleotide probes designed in this study to develop PCR-probe hybridization assay for Mycobacterial species identification.

Name of oligonucleotides	Sequences of oligonucleotides	<u>T</u> ŧarget Mycobacteria
PAN-MYC	GACGTCGTCGCCACCATCGA	All mycobacterial species
	(nucleotides 108 to 127 of SEQ ID NO:1)	
TB	CATGTCGGCGAGCCC	M. tuberculosis complex
	(nucleotides 66 to 80 of SEQ ID NO:5)	

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Name of oligonucleotides	S quences of oligonucleotides	<u>T</u> ŧarget Mycobact ria
AVIUM	CGGTGAGCCGATCACCA	M. avium
	(nucleotides 71 to 87 of SEQ ID NO:15)	
INTRA	CCTGCACGCGGCGA	M. intracellularae
	(nucleotides 62 to 76 of SEQ ID NO:20)	
GORDONAE	GTCGGCGATCCGATCA	M. gordonae
	(nucleotides 69 to 84 of SEQ ID NO:1)	
SZULGAI	TCTGAACGTCGGCGAG	M. szulgai
	(nucleotides 61 to 76 of SEQ ID NO:12)	
KANSASII	GGCCACGATGACCGTG	M. kansasii
	(nucleotides 155 to 170 of SEQ ID NO:8)	
GASTRI	TCTGAACGTCGGCGAG	M. gastri
	(nucleotides 61 to 76 of SEQ ID NO:12)	
FORTUITUM	CCTGAACGCCGGCCAG	M. fortuitum
·	(nucleotides 62 to 77 of SEQ ID NO:19)	
FORTUITUM-COM	GTTCCGGTCGAGGTGG	M. fortuitum complex
SCROFULACEUM	CGTACGGATGGCCAGC	M. scrofulaceum
	(nucleotides 153 to 168 of SEQ ID NO:9)	
CHELONAE	TGGTGACTGCCACCACG	M. chelonae
	(nucleotides 85 to 101 of SEQ ID NO:7)	
ABSCESSUS	AGGTGACCACCACC	M. abscesus
	(nucleotides 85 to 101 of SEQ ID NO:21)	
TERRAE	GCTCAGGACGGTCAGT	M. terrae
ULCERANS/	GGCCAGCCCATCACC	M. ulcerans / M. marinum
MARINUM	(nucleotides 72 to 86 of SEQ ID NO:10)	
GENAVANSE/SIMIAE	CCAGCCACGATGACG	M. genavanse / M. simiae

Paragraph spanning pages 16-17:

PCR-reverse blot hybridization. All oligonucleotide probes to be applied on the membrane were synthesized with 5' terminal amino group, which link the oligonucleotides to the Biodyne BIODYNE® C membrane (Pall BioSupport, East Hills, NY) by forming covalent bond with negatively charged carboxyl group fixed on the

membrane. Before blotting the oligonucleotide probes, the Biodyne BIODYNE® C membrane was activated by incubating in 10 ml of freshly prepared 16% (w/v) 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDAC). After rinsed with the water, the membrane was placed on a support cushion in a clean miniblotter system (Immunetics, Inc., Cambridge, MA), and the residual water was removed from the slots. Then, the slots were filled with 150 μ l of the diluted oligonucleotide solutions (approximately 200 pmol to 1 nmol of oligonucleotides in 150 μ l of 50 mM NaHCO₃, pH 8.4). Subsequently, the membrane was incubated for 1 hr at room temperature, and then excess amount of oligonucleotide solution was removed from the slots by aspiration. In order to inactivate the membrane, the membrane was removed form the miniblotter using forceps, incubated in 100 mM NaOH for 10 min in a rolling bottle, and washed in 100 ml 2x SSPE/0.1% SDS for 5 min at 60°C in a plastic container under gentle shaking. Before applying PCR products on the Biodyne BIODYNE® C membrane, the membrane was incubated for 5 min at room temperature in 100 ml 2x SSPE/0.1% SDS.

IN THE CLAIMS

The claims are amended as follows.

- 1. (Amended) A DNA fragment which has one of the sequences a sequence selected from the group consisting of SEQ Seq. ID. NOS:1 to 4 and or 6 to 24.
- 2. (Amended) A method of diagnosis and identification Mycobacterium strain for identifying the species or subspecies of a mycobacterial strain comprising the steps of:
- a) digesting a DNA fragment which has ene a sequence selected from the group consisting of the sequence Seq ID. NO 1 to 4 or 6 to 24 SEQ ID NO:1 to SEQ ID NO:24 with at least one restriction enzyme selected from the group consisting of Haelll, Mspl, Sau3AI, and BstEll to obtain a first DNA fragment length polymorphism pattern;
- b) isolating <u>a</u> DNA fragment from microorganisms the mycobacterial strain to identify be identified;

- c) amplifying said rpoB region of the DNA fragment isolated in step (b), said amplification being performed by using a primer of SEQ ID NO: 15 or SEQ ID NO: 26;
- d) digesting said-amplified the DNA fragment amplified in step c) with the at least one same restriction enzyme employed in step a) to obtain a second DNA fragment length polymorphism pattern; and
- e) obtaining DNA fragment length polymorphism pattern from DNA fragment in step d); and
- f) e) comparing the first DNA fragment length polymorphism pattern obtained in from step a) with the second DNA fragment length polymorphism pattern obtained in from step e) step d), thereby identifying the species or subspecies of a mycobacterial strain.
- 3. (Amended) A method of claim 2, wherein said <u>first and second</u> DNA fragment length polymorphism from stop a) and stop 3) are characterized as obtaining patterns are obtained by electrophoresis.
- 4. (Canceled)
- 5. (Amended) A method of claim 2, wherein said Mycobacteria mycobacterial strain is selected from the group consisting of M. tuberculosis, M. avium, M. abscessus, M. flavescence flavescens, M. africanum, M. bovis, M. chelonae M. chelonae, M. celatum, M. fortuitum, M. gerdenae, M. gordonae, M. gastri, M. gastri, M. haemophilum, M. intracellulare, M. kansasii, M. malmoense, M. marinum, M. szulgai, M. terrae, M. scrojulaceum, M. ulcerans, er and M. xenopi.

IN THE DRAWINGS

Corrected Figs. 6a-6b are attached.



Morked- WP Copy of Corrections

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M.gordonaely TCHRGGAGHAGGG-TRCGACCTGTGGGCCCTGTARAGGTCARCAGGGGGCTGGCTGCTGTTCGGCGHTCGHTGATCHAGGTCARGGTTGHAG	TCBAGGAGAAG	CGC-TACGACCT	GGCCGTGTCGG	CCCTACA	GETCAACAAG	MGC1666CCT	C8T6T-	-CGGCGATCCG	TCACC	ACCTCGAC-	-GCTGACCGANG	
M.gordonael TCRRGGGRAGGCC-TRCGRCCTGGCCCGTGTCGCCCTGTCACACACACACACACACACA	TCARGGAGARGI TCARGGAGARGI	SGC-TACGACCT SGC-TACGACCT	GGCCCGGGIMGG GGCCCGTGTCGG	CECTRCA	GGTCAACAAG	HOLI COBCCT	CRCGT	M.gordonael TCARGGAGARGEGE-TACGARCTGGACCTGGACGGACACATACATAGATAGACACACATACACATACACATACAGATACAGACACAGAGACAGAGACAG gordonaelli TCARGGAGARGEGE-TACGACCTGGACCGTGTCGACAGACACAGACAGACAGACA			-6CTGACCGARG	
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M.ulcerans	TCARGERGRAG	CGC-TRCGRCCT	GECTCECCTEGE	TCGGTACAR	GETCARCARG	MGCTCGGCCT	HACGC-	NULGERAGE TERREGERRECCE-TRUBCHCTGGCTCGCGTGGGTCGGTGCGTGAGGTCARGGTCGGCCTGRACGCCGGCCRGCCATCACTCATCACTCACTCACTCACTCACTCACTCACT	33-31E	-AGCTCGAC-	-6CTGACCGAGG	
M.fortuitum	TCARGERGARE	CEC-TACGACCT	990193030399	CECTROR	GETCHRCHRE	HGCTGGGCCT	999	n.Natutum ichnommouc-nichnet nateleeren nateleeren materiannan ichnommoneren eren den der	TCRC6	-TCGTCGRC-	-TCTGACCGAGG	
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